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The Biology of Bone Metastasis

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Summary

Bone metastasis, or the development of secondary tumors within the bone of cancer patients, is a debilitating and incurable disease. Despite its morbidity, the biology of bone metastasis represents one of the most complex and intriguing of all oncogenic processes. This complexity derives from the intricately organized bone microenvironment in which the various stages of hematopoiesis, osteogenesis, and osteolysis are jointly regulated but spatially restricted. Disseminated tumor cells (DTCs) from various common malignancies such as breast, prostate, lung, and kidney cancers or myeloma are uniquely primed to subvert these endogenous bone stromal elements to grow into pathological osteolytic or osteoblastic lesions. This colonization process can be separated into three key steps: seeding, dormancy, and outgrowth. Targeting the processes of dormancy and initial outgrowth offers the most therapeutic promise. Here we discuss the concepts of the bone metastasis niche from controlling tumor cell survival to growth into clinically detectable disease.

Keywords

Bone metastasis; Hematopoietic Stem Cell (HSC) niche; vascular niche; osteoblast niche; vicious cycle

Introduction

The most immediate and pressing concern upon receiving a cancer diagnosis is to determine the extent to which it has spread – a process known as cancer metastasis. Metastasis to any organ presents a life-threatening and often incurable disease, yet among the different organs that host metastatic disease, the biology of bone metastasis is perhaps the most exceptional. The first unique aspect is the “reverse hematopoiesis” that takes place; during normal hematopoiesis, hematopoietic stem cells mature into the variety of cell types that form the blood and enter into circulation. During the systemic spread of cancer, this process appears to run in reverse wherein the spongy tissue of the red marrow can collect disseminated tumor cells (DTCs) from nearly any type of cancer (Aguirre-Ghiso 2007) and the nurturing niche that normally hosts HSCs is hijacked by the cancer cells (Shiozawa et al. 2011). This idea is supported by the broad clinical observation that bone metastasis only forms in sites that host hematopoietically-active red marrow (Kricun 1985). A second unique feature of bone metastasis is the subversion of the biological processes that are responsible for the structure

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of bone- both osteolysis and osteogenesis. Considerable research efforts have focused on the unique ability of certain cancers to grow in bone by destroying the calcified matrix while others appear to grow via generating new bone tissue (Mundy 2002).

Research efforts aimed at discovering new therapies for patients with bone metastasis have almost exclusively focused on three mechanisms that can be thought of as the minimum essential requirements for forming bone metastasis. These are 1) Seeding to the bone, 2) Survival via dormancy, and 3) Eventual outgrowth into osteolytic or -genic tumors. These efforts have led to the approval of two therapies that attenuate cancer-induced osteolysis as well as diagnostic tools that detect dormant cancer cells in the bone marrow (Esposito and Kang 2014). However, a cure for this pathology cannot be achieved until the fundamental drivers of bone metastasis formation are discovered, particularly the drivers of survival during dormancy or exit from this state. Given the clinical importance of bone metastasis, the following chapter will focus not only on the well-established mechanisms of bone metastasis but also areas of study that are likely to yield new clinical strategies to treat bone metastasis.

Characteristics of primary tumors that form bone metastasis

The unique predilection of certain tumors to form bone metastasis while others cannot, despite sharing similar circulatory patterns and tumor cell deposition in the bone matrix, is indicative that either the cell of origin or stromal influence at the primary tumor is essential for bone metastasis. There is also ample evidence to support the idea that inducers of cellular plasticity, cancer stemness, and the epithelial to mesenchymal transition (EMT) primes certain cells for bone metastasis (Kang and Pantel 2013). An illustration of the importance of the cell of origin is that healthy kidney tissue normally expresses high levels of the calcium-sensing receptor (CsR) in order to control calcium homeostasis in blood. Analysis of renal cell carcinoma (RCC) patients showed a strong correlation of CsR with bone metastasis, and calcium addition to RCC cells was mitogenic in cells from patients with bone metastasis (Joeckel et al. 2014). Supporting the idea that stromal influences precede bone metastasis, a study revealed that cancer-associated fibroblasts secrete CXCL12, thereby priming the tumor cells for metastasis to organs rich in CXCL12 via selection for high SRC activity (Zhang et al. 2013). Finally, support for the necessity of stemness/EMT is demonstrated by the finding that embryonic miR409 correlated with higher Gleason score and EMT/stemness gene signatures in prostate cancer tumors (Josson et al. 2014) or that a loss of PTEN and gain of RAS/MAPK signaling in prostate cancer induced an EMT that resulted in bone metastasis with 100% penetrance (Mulholland et al. 2012).

Even within cancer types with a strong propensity to develop bone metastasis, different clinical subtypes manifest bone metastasis to vastly different degrees, thereby demonstrating that neither stromal influence nor site of origin can fully predict the mechanisms of bone metastasis. The most notable example is breast cancer bone metastasis, where a 15-year cumulative assessment 1357 patients with metastatic disease showed that while bone is the most common specific site of relapse for all but one subtype, bone metastasis is observed in 71% of patients with Estrogen Receptor-positive (ER+) tumors and 47% of patients with ER – tumors (Kennecke et al. 2010)). Furthermore, patients with ER– tumors tend to manifest

overt metastatic disease within 5 years of diagnosis while those with ER+ tumors experience a steady rate of presentation up to 10 years after (Kennecke et al. 2010). While this observation indicates that bone metastasis is of paramount concern to patients with all breast cancer subtypes, it indicates that the mechanisms of bone metastasis differ greatly even within one type of cancer.

Metastatic dissemination to bone

Passive shedding to the bone and vascular entry

While specialized seeding mechanisms may be required for brain metastasis (Valiente et al. 2014), lung (Gupta et al. 2007; Padua et al. 2008) and other organs, multiple lines of evidence support the idea that tumor seeding to the bone marrow may not require specialized processes but is rather the result of passive entry. This may be in part due to fact that the bone sinusoids have a discontinuous endothelium to facilitate the passage of hematopoietic and other cells (Oghiso and Matsuoka 1979), in contrast to the tight cell-cell junctions and continuous endothelium of the lung and other organs. In a landmark study, bone metastasis-competent DTCs were detected in the bone marrow of mice harboring only atypical ductal hyperplasia or ductal carcinoma in situ (Hüsemann et al. 2008). While metastasis is typically characterized as the final step of primary tumor growth, this study demonstrates that primary tumors and metastatic lesions can develop in parallel rather than in sequence; a hypothesis that is further supported by the fact that DTC status is not correlated to tumor size (Hüsemann et al. 2008). This finding was clinically validated by the detection of DTCs in the bone marrow of patients diagnosed only with breast ductal carcinoma *in situ* (DCIS) (Sänger et al. 2011; Banys et al. 2012) or localized Prostate cancer (Melchior et al. 1997).

Even further supporting the idea that tumor seeding into the bone marrow is not a selective process, 32% of colorectal cancer patients harbored cytokeratin-positive bone marrow smears after radical resection, yet colorectal cancer rarely forms bone lesions and none of the patients in this study developed clinical bone metastases despite relapsing at other sites (Lindemann et al. 1992). Despite this evidence that DTCs do not always develop into bone metastases (Melchior et al. 1997), meta-analyses of all studies of prognostic indicators of breast cancer relapse established the presence of cytokeratin-detectable DTCs as a highly significant predictor of relapse to bone (Braun et al. 2005). The integration of these multiple lines of evidence therefore indicates that passive dissemination of tumor cells to the bone marrow is the early step in forming bone metastasis, but is not the critical driving event of bone metastasis formation.

Maintenance within the bone

On the other hand, numerous molecular mechanisms have been described that promote residency within the bone, potentially through chemotaxis to specialized bone niches. Among the characterized interactions, that between SDF1/CXCL12 and CXCR4 is the best studied, both in bone metastasis and HSC function (Teicher and Fricker 2010). Early studies showed that breast cancer cells typically express both CXCR4 and CCR7, while their respective ligands, SDF1 and CCL21, are expressed in sites that commonly host breast cancer metastases (Muller et al. 2001). Furthermore, CXCR4 is one of the most enriched

genes following *in vivo* selection for highly bone metastatic breast cancer variants (Kang et al. 2003). This mechanism is shared with HSCs as transgenic expression of CXCR4 *ex vivo* promotes better engraftment of HSCs into the bone marrow (Brenner et al. 2004; Kahn et al. 2004). Furthermore, G-CSF/GM-CSF is the standard treatment used to mobilize HSCs into the blood for collection and transplantation, which occurs via specific down-regulation of SDF1 within human bone marrow (Petit et al. 2002). In a parallel manner, prostate cancer cells compete with HSCs for the same niche, and treatment with G-CSF can mobilize these prostate cells into circulation (Shiozawa et al. 2011). Treatment with AMD3100, a CXCR4 antagonist, can also mobilize AML cells from the bone into the blood where they become more sensitive to chemotherapy (Nervi et al. 2009). Further studies show that inhibition of the CXCR4 interaction between cancer cells and the stroma sensitizes these cells to standard chemotherapy (Domanska et al. 2012), pointing toward the pleiotropic role of this interaction. More targeted studies of the interaction show that specific deletion of SDF1 in vascular endothelial cells of the bone marrow is sufficient to impede T cell acute lymphoblastic leukemia growth, implying that a SDF1-producing vascular niche is critical to bone metastasis progression (Pitt et al. 2015).

Pharmacologic inhibition of PDGFR on bone marrow-derived cells with Sunitinib has also implicated PDGFR in homing or maintenance within bone, but the exact mechanism is unclear (Catena et al. 2010). The functions of receptor activator of NF- κ B (RANK) and its ligand (RANKL) also extend beyond their canonical role in osteoclastogenesis (covered below) as RANK is expressed by hormone-responsive epithelial cancers, namely breast and prostate cancer. This expression was instructive in guiding migration along RANKL gradients *in vitro*, and growth of non-osteolytic bone metastases were slowed by OPG treatment *in vivo* (Jones et al. 2006). Therefore, cancer cells expressing RANK may be attracted to sites of active osteolysis where high concentrations of RANKL and other fostering stromal components exist.

Survival and dormancy in the bone niche

Clinical evidence for dormancy

Of all the anatomical compartments in which disseminated tumor cells have been detected, the bone marrow appears to be the only site that harbors minimal residual disease from nearly every cancer type (Aguirre-Ghiso 2007), yet the majority of these cancer types will never develop bone metastases. For those cancer types that do often develop bone metastases, such as prostate or breast cancer, DTCs in the bone marrow are detected at much higher rates than metastatic disease develops (Harbeck et al. 1994; Melchior et al. 1997). This observation indicates that while many cancer types are competent to enter a dormant state in the bone, the control of and emergence from dormancy may be the keystone variable in predicting metastatic relapse.

The duration of dormancy is widely variable; breast cancer patients diagnosed with Luminal A/B tumors experience a steady probability of metastatic relapse to bone for 10 years following diagnosis while those with the more aggressive TNBC subtypes develop bone metastases within 5 years of diagnosis (Kennecke et al. 2010). Further complicating the study of dormancy, it is unclear if metastatic latency is controlled by cell autonomous

programs or by the bone metastasis “niche” (Ren et al. 2015). Understanding the molecular basis of this dormancy as well as the factors influencing the exit from dormancy will be instrumental to lowering the rate of bone metastasis-associated deaths.

Dormancy and survival inducing cellular programs in HSCs and cancer

Several cell autonomous means of DTC survival during dormancy have emerged, most relating to various types of stress responses. Early reports provided evidence that early DTCs found in the bone marrow of breast cancer patients showed a high enrichment of mesenchymal-like CD44+/CD24– cells compared to the primary site (Balic et al. 2006), therefore indicating that EMT is a critical process for early dissemination and survival. Further supporting this idea, data from Malladi and colleagues has shown that early DTCs exhibit a mesenchymal phenotype and maintain high DKK1 levels in order to inhibit active Wnt signaling and thereby evade immune surveillance (Malladi et al. 2016). These DTCs are also resistant to conventional cytotoxic therapy, as shown by positive cytokeratin staining in bone marrow aspirates of advanced breast cancer patients after standard of care chemotherapy (Braun et al. 2000). A combined clinical and experimental analysis showed that Src expressed by latent breast cancer cells is a central positive regulator of DTC survival in response to the stromal environment (Zhang et al. 2013).

Stromal control of dormancy in the HSC and bone metastasis niche

While bone metastatic tumor cells have been ascribed with a few cell-autonomous means of maintaining dormancy during the bone metastatic process, much more evidence indicates that the metastatic “soil” controls dormancy through both context- and temporally-dependent cues from the surrounding stroma. This idea is exemplified by a study of breast cancer dormancy in multiple organ sites wherein dormancy was maintained by vascular contact and communication via thrombospondin-1, but sprouting of neovasculature released TGFβ and periostin, which stimulated proliferation of dormant cells (Ghajar et al. 2013). Other studies have found that the stromal compartment induces the p38 stress response via secretion of BMP7, which is bound by BMPR2 found on prostate cancer cells. This result was interpreted as an innate host defense mechanism (Kobayashi et al. 2011). A third model described a situation where high TGFβ2 concentrations in the bone caused tumor dormancy while lower concentrations in the lung were permissive to growth (Bragado et al. 2013). A similar mechanism was found wherein KAI1/CD82 is inversely correlated with prostate cancer bone metastases, and mechanistic analysis revealed that its binding to DARC in the bone marrow causes p21-induced dormancy (Bandyopadhyay et al. 2006). In cases where the stroma enforces a state of dormancy within the tumor cells, such as FGF-induced growth arrest, adhesion to extracellular matrix proteins such as Fibronectin (FN1) may be instructive in maintaining cell survival via integrin β1 binding (Korah et al. 2004). Supporting the necessity of adhesion to the niche components, one study showed that heterotypic adherence junctions between tumor cell E-cadherin (CDH1) and osteoblast N-cadherin (CDH2) are critical to the early colonization of breast cancer cells (Wang et al. 2015) (Figure 1).

While the molecular cues that control tumor cell dormancy remain largely uncharacterized, a greater body of evidence describes the regulation of HSC dormancy. At the center of this research is a complete description of the HSC niche in both the active and quiescent state

(Morrison and Scadden 2014). A series of experiments seeking to understand the physical components of the HSC niche and how those influence HSC maintenance and expansion have uncovered a critical role for osteoblasts (Zhang et al. 2003). Specifically, HSCs and osteoblasts physically interact in the bone niche at the endosteal surface, and treatment with parathyroid hormone (PTH) stimulates HSC expansion in what is assumed to be an osteoblast-dependent manner (Adams et al. 2007). Follow-up studies have since established that HSCs and OBs are co-localized the endosteal surface, where OB stimulation with PTH induces Jagged1 expression, which in turn activates Notch signaling in HSCs to promote their proliferation (Calvi et al. 2003). Physical contact between HSC and OB cells has also been shown to maintain HSC quiescence via TIE2 and ANGPTL interaction (Arai et al. 2004). In addition to osteoblasts, endothelial cells are the other major component of the HSC niche, with two studies demonstrating that SDF1 and SCF expressed by endothelial cells and perivascular stromal cells are both essential for HSC maintenance (Ding et al. 2012; Ding and Morrison 2013). Another study showed that E-selectin expressed by endothelial cells in the vascular niche bound to HSCs and induced proliferation and/or differentiation (Winkler et al. 2012). Other components of the HSC niche have also been described to a lesser extent. One study showed that MSCs are an essential regulatory element of the HSC niche as Nestin depletion concomitantly reduces HSC residency (Méndez-Ferrer et al. 2010). On the other hand, the matrix protein Osteopontin (OPN) is a negative regular of HSC pools (Stier et al. 2005).

The application of these findings to the bone metastasis field has already yielded new therapeutic opportunities and demonstrated that bone metastatic cells mimic HSCs to a large extent. For example, interactions between Jagged1-Notch1, SDF1-CXCR4, and GM-CSF-FMS are at the center of our current understanding of bone metastasis. Therefore, additional research into the applicability of these other uncharacterized interactions, such as the role of Tie2, SCF, or E-selectin, may yield valuable insights into the regulation of bone metastatic cells in the HSC niche (Figure 1).

Role of the immune system in dormancy

Avoidance of immune surveillance in early bone lesions is an essential but overlooked component of bone metastasis. Limited experiments conducted thus far indicate the immune system limits bone metastasis colonization and enforces dormancy. Despite the observable immune privilege of the bone marrow HSC niche (Fujisaki et al. 2011), CD8+ T cells actively control proliferation and enforce dormancy of lymphoma cells (Muller et al. 1998). Clinical analysis of bone marrow aspirates from breast cancer patients found the highest proportions of CD56+ CD8+ T cells and memory CD4+ T cells in patient marrow samples harboring DTCs compared to either tumor-bearing but DTC-negative patients or healthy donors (Feurerer et al. 2001). More recent studies have shown that dormant DTCs express DKK1 to suppress Wnt signaling and therefore proliferation-associated antigens, providing a mechanism of immune escape from Natural Killer cells (Malladi et al. 2016). Further research has also shown that Interferon-induced genes are strongly silenced in patients with spine metastasis (Bidwell et al. 2012). At the same time, components of the adaptive immune system have also shown important roles in establishing immune suppression and facilitating bone metastasis. For example, infiltrating plasmacytoid dendritic cells (pDC)

establish a sustained Th2 response that suppresses CD8+ T Cell function and promotes Treg maturation. In turn, depletion of pDCs slows bone metastasis progression by promoting the expansion of cytolytic CD8+ T cells (Sawant et al. 2012).

From colonization to the vicious cycle of metastatic expansion in bone

Triggering outgrowth

The events taking place between the seeding on DTCs in the bone marrow and the emergence of clinically-detectable bone lesions are the least characterized yet most important molecular interactions of the bone metastatic cascade. This deficiency can be traced to the fact that no *bona fide* experimental models of bone metastasis dormancy exist, particularly when mouse models that cause death within 3–6 weeks are compared to the multi-year pathogenesis observed in patients. Limited studies performed thus far have implicated several interactions in driving tumor outgrowth, but the discovered pathways are by no means exclusive, and research into tumor dormancy will revolutionize our understanding of metastasis in the coming years,

From the limited work that has been performed, three major developmental signaling programs appear to be the master regulators of emergence from bone metastasis dormancy: TGF β , Wnt, and Notch. Multiple studies have demonstrated that the TGF β signaling pathway is active throughout the full course of bone metastasis, but its role in dormancy exit was revealed by the finding that pharmacologic inhibition was only effective in lessening bone metastasis burden when treatment commenced 3 days post-injection compared to 21 days post-injection (Korpai et al. 2009). This study further suggested that TGF β signaling, tumor cell growth, and osteoclastogenesis are functionally related as bisphosphonate treatment, by blocking osteoclastic bone resorption, also reduced TGF β activity by preventing release and activation of TGF β from the mineralized bone matrix. (Korpai et al. 2009). Wnt signaling has been implicated but not proven to be a driver in bone metastasis, as Wnt suppression via DKK1 is critical to maintaining metastatic dormancy, suggesting that Wnt activation is an early event in the eventual outgrowth of metastatic lesions (Malladi et al. 2016). Of the three implicated signaling pathways, Notch activation appears to be the most important in early colonization events as well as advanced osteolysis (Zayzafoon et al. 2004; Sethi et al. 2011).

Broader transcriptional identity is also important to the early events of bone metastasis; considerable evidence suggests that EMT and stemness are essential to early DTC survival (Kang and Pantel 2013; Malladi et al. 2016). Yet, markers of epithelial cells are used to detect bone micrometastases (Mansi et al. 1987), thus a mesenchymal to epithelial transition (MET) must accompany the outgrowth of metastatic lesions. Given that multiple developmental pathways are closely related to the balance between EMT/MET and stemness (Thiery and Sleeman 2006), considerable work must be performed to understand how these signaling pathways instruct tumor cell identity.

Beyond the major signaling programs, numerous adhesion proteins and chemokine signaling programs are implicated in dormancy exit. Tumor cell Integrin $\alpha 4 \beta 1$ was one of the original adhesion proteins found to be important for bone metastasis (Matsuura et al. 1996), and its

engagement to VCAM1 expressed by stromal cells stimulates osteoclast cells (Michigami et al. 2000). Studies have since found Integrin $\alpha 4 \beta 1$ to be expressed on multiple stromal components of the bone with varying functions. For example, the interaction between NF- κ B-induced VCAM1 on dormant breast cancer cells and Integrin $\alpha 4 \beta 1$ expressed on monocytes can regulate the exit from dormancy by elevating local osteoclast activity (Lu et al. 2011). Regardless of the expression patterns, depletion of $\alpha 4$ reduces myeloma-associated osteolysis (Mori et al. 2004), while attachment to the bone matrix has been observed in multiple bone metastasis models via integrin $\alpha 2 \beta 1$ (Hall 2006).

While many studies suggest that an amplification of osteolytic activity is necessary for initial outgrowth, some evidence also suggests that there is decoupling between the bone degradation processes and the emergence from dormancy. Osteoclast-specific deletion of either $\beta 3$ integrin or Src does not affect tumor cell proliferation in the bone but does prevent osteolysis (Bakewell et al. 2003). Given that Src was previously shown to be essential for osteolysis (Boyce et al. 1992; Schwartzberg et al. 1997), this study demonstrates that osteoclasts are not strictly required for the early outgrowth events. One potential confounding detail of this research is that inducers of dormancy exit are difficult to distinguish from generally mitogenic mechanisms within the bone marrow. For example, Lypophosphatidic acid derived from platelets and bound by bone metastatic breast cancer cells causes growth (Boucharaba et al. 2004), but the relevance to dormancy is unknown.

Vicious cycle of osteolytic bone metastasis

Once metastatic cells have established a foothold within the bone niche, they become growth-limited by multiple factors – nutrients, growth factors, oxygen delivery and physical space. For cancer types that form osteolytic metastases, such as hormone independent-breast cancer and multiple myeloma (Mundy 2002), these constraints are solved by a unique positive feedback cycle composed of tumor cells, osteoclasts, osteoblasts and the bone matrix. This “vicious cycle of bone metastasis” has been extensively studied (Roodman 2004; Weilbaecher et al. 2011) and is targeted by the only FDA-approved bone metastasis therapies: the bisphosphonates zoledronic acid and pamidronate (Zometa and Aredia, Novartis); the anti-RANKL antibody, denosumab (Denosumab, Amgen) and a radionucleotide, radium-223 (Xofigo, Bayer).

The core vicious cycle initiates when tumor cells stimulate osteoclastic bone resorption. TGF β released from the mineralized bone matrix as a consequence of osteoclastic bone resorption binds to these bone metastatic tumor cells, which subsequently activates expression of osteolytic factors such as PTHrP (Yin et al. 1999) and Jagged1 (Sethi et al. 2011). Jagged1 promotes osteoclastogenesis by directly binding monocytes while PTHrP binds osteoblasts and induces the production of RANKL (TNFS11) (Boyle 2003). RANKL is the major driver of osteoclast differentiation from monocytes (Anderson et al. 1997; Yasuda et al. 1998) and its binding to RANK is sufficient to induce osteoclast maturation from committed hematopoietic precursors (Lacey et al. 1998). RANKL binding to RANK induces NF- κ B signaling, which is essential for osteoclast formation (Iotsova et al. 1997). Activated osteoclasts then degrade the surrounding bone matrix, resulting in the release of numerous mitogenic growth factors, such as TGF β , which further fuels this feed-forward

cycle (Hauschka et al. 1986) (Figure 2). TGF β -induced Jagged1 further enhances this vicious cycle by stimulating the production of tumor growth-promoting IL-6 from osteoblasts (Figure 2).

Beyond this core cycle, many additional regulators and interactions have been described, both during bone metastasis and normal development (Boyle et al. 2003). One of the first discovered factors was Osteoprotegerin (OPG), a protein that serves as a decoy receptor for RANKL whose administration prevents breast cancer-induced osteolysis in experimental models (Morony et al. 2001). Additional osteoclastogenesis-stimulating factors have been discovered beyond RANK-RANKL and Jagged1-Notch, the most significant of which is the CSF1-FMS interaction (Park et al. 2007).

Each core component of the vicious cycle also has pleiotropic roles in regulating osteolysis. For example, PTHrP secretion also induces CCL2 production in both endothelial and osteoblast cells of the bone marrow (Li et al. 2009). A study using mouse versus human-specific anti-CCL2 antibodies suggested that CCL2 derived both from the tumor cells and the stroma are responsible for bone metastasis progression (Loberg et al. 2007).

Several classes of proteases and extracellular matrix-modifying enzymes have been implicated in the osteolytic cascade. For examples, ADAMTS1 and MMP1 were shown to release growth factors from the matrix of tumor cells, and these were in turn responsible for potentiating osteoclast signaling (Lu et al. 2009). Osteoclast-derived MMP7 has also been implicated in a similar process through solubilization of RANKL (Lynch et al. 2005). Conversely, expression of Tissue-inhibitor of metallo-proteases (TIMP2), or Maspin, an inhibitor of uPA, prevents bone metastasis or proteolytic remodeling of bone fragments implanted with prostate cancer (Yoneda et al. 1997; Cher et al. 2003). Tumor cell secretion of Heparanase (HPSE) can distantly promote osteolytic degradation through an unknown mechanism (Kelly et al. 2005) and hypoxia induction in ER- tumors has also been shown to enhance LOX secretion, which can independently activate the osteolytic process (Cox et al. 2015). Finally, Cathepsin was implicated as an additional collagen-degrading enzyme essential for a spontaneous bone metastasis model (Withana et al. 2012).

Numerous methods to treat this vicious cycle have also emerged, in most cases derived from osteoporosis therapies. The earliest of these approaches used bisphosphonates in preclinical models, resulting in the FDA approval of pamidronate (Aredia) as the first bisphosphonate used to bone metastases for breast cancer or multiple myeloma patients. This success was followed by the approval of zoledronic acid (Zometa) to treat bone metastasis from all solid tumors or myeloma. Bisphosphonates function by binding to the mineralized bone matrix and inhibiting enzymes of the mevalonate pathway when internalized in osteoclasts. More recently, blockade of the RANKL pathway with Denosumab has emerged as the most effective anti-resorptive treatment for bone metastases (Esposito and Kang 2014). Another approach to prevent bone resorption is treatment with OPG, the decoy receptor for RANKL, whose administration reduces osteolysis *in vitro*, *in vivo*, and in patients (Body et al. 2003; Canon et al. 2008).

Beyond the direct targeting of osteoclasts, subsequent studies have also suggested that the bisphosphonates may have direct tumor-killing properties, albeit at concentrations much higher than those observed *in vivo*. It was therefore suggested that local concentrations of bisphosphonate may kill tumor cells in areas of active osteolysis while also providing a rationale for the lack of efficacy on primary tumors (Hiraga et al. 2001). Other discoveries have found that pharmacologic blockade of both TGF β and HIF1 α were additively effective in treating bone metastasis while genetic ablation of these pathways in tumor cells showed redundancy (Dunn et al. 2009). Multiple studies have also developed kinase inhibitors of the FMS receptor, which show promise in treating osteolytic bone metastasis (Murray et al. 2003; Ohno et al. 2006). Additionally, the well-characterized cycle of bone degradation does offer a silver lining in that it offers multiple means of progression monitoring, particularly in clinical trials. For example, N-telopeptide of collagen (NTX) can be used as a biochemical marker to monitor the efficacy of anti-resorptive agents (Body et al. 2003).

Recently, alpha-emitting radionucleotides, such as Radium-223, have been developed to treat osteoblastic bone metastases from prostate cancer. This agent targets bone forming activity and has been shown to improve overall survival in prostate cancer patients with bone metastases (Parker et al. 2013; Hoskin et al. 2014; Sartor et al. 2014). Preclinical data indicate that such therapy may also be effective in treating osteolytic bone metastases (Suominen et al. 2013).

Osteoblastic metastasis

In comparison to the well-researched mechanisms of osteolytic metastasis, the molecular processes that give rise to osteoblastic metastasis are relatively unexplored. One clear finding is that osteolytic activity is still unbalanced in osteoblastic metastases; NTX is highest in prostate cancer patients with osteoblastic disease, compared to patients with osteolytic bone metastases (such as breast cancer). Furthermore, increased NTX predicts higher overall morbidity (Coleman et al. 2005) and death in patients with prostate cancer (Brown et al. 2005). In fact, NTX was more significant predictor of death than prostate-specific antigen (PSA) in this setting (Brown et al. 2005). Several osteoblast-exclusive signaling programs have also been discovered. One mechanism that increases bone mineralization via increased OB activity is secretion of BMP2 or BMP6 by metastatic prostate cancer cells (Dai 2005). Another interesting clue giving insight into the pathogenesis of osteoblastic bone metastasis is the well-validated role of RUNX2 in promoting both breast and prostate bone metastasis (Akech et al. 2010; Baniwal et al. 2010). RUNX2 is a transcription factor found in mature osteoblasts (Lee et al. 2000) that was shown to exert pleiotropic roles necessary for the metastatic process (Baniwal et al. 2010). Osteoblast cadherin (CDH11) was also validated as an important stromal interaction protein in prostate cancer (Chu et al. 2008), further supporting the idea that osteoblastic cancer cells mimic osteoblast expression programs. Studies have also suggested that DKK1 is the decisive factor in determining the balance between tumor-induced osteolysis vs osteogenesis. In one study, DKK1 depletion promoted exclusively osteolytic lesions while supplementation converted to exclusively osteoblastic metastasis (Hall et al. 2005). However, the reverse scenario was observed in multiple myeloma patients, where DKK1 inhibited osteoblast maturation (Tian et al. 2003).

This process of osteolytic destruction or osteogenesis doesn't only engage and affect the balance of OB and OC cells. Recent findings have also established that the vicious cycle is responsible for suppressing HSC differentiation into each of the different lineages (Bruns et al. 2012) as well as affecting the number and quality of HPCs (Colmone et al. 2008). This HSC/HPC suppression was attributed to increased TGF β signaling that reduced the plasticity of HSC and HPCs by broadly changing adhesion and other niche interactions (Bruns et al. 2012).

Resistance to current therapies

Bone metastases exhibit peculiar resistance mechanisms that were not anticipated *a priori*, but are rather caused by crosstalk between the metastatic cells and the bone stroma. At the center of this is the interaction between steroid hormones, OB/OCs and tumor cells.

Despite broad use of bisphosphonates as highly effective bone metastasis therapies, an early meta-analysis of bisphosphonate treatment concluded that bisphosphonate treatment did not significantly benefit patients (Ha and Li 2007). A second meta-analysis performed a decade later deconvoluted this result by demonstrating that bisphosphonate treatment is only effective in preventing skeletal-related events in patients who were post-menopausal when treatment began (Coleman et al. 2015). This was experimentally validated by a study demonstrating that Zoledronate slowed osteolysis only in ovariectomized mice compared to sham when injected with a triple negative breast cancer line, thereby mimicking the post-menopausal state and showing that Zoledronate is only effective in low estrogen individuals (Ottewell et al. 2014a). Many of these observations can be explained by the finding that free estrogen binding to ER α causes apoptosis in osteoclasts in females (Nakamura et al. 2007), therefore indicating that estrogen and bisphosphonates may act redundantly. However, free estrogen or androgens are mitogenic for breast cancer and prostate cancer cells, and depletion of free steroid levels is a mainline therapy for most patients (Dowsett et al. 2015). Thus a paradox emerges wherein aromatase inhibitors reduce free estrogen levels and therefore breast cancer cell growth yet contribute to increased osteoclast activity (Smith and Dowsett 2003). In this setting, complete estrogen deprivation with ovariectomy and aromatase inhibitors increases bone resorption and bone loss, thereby stimulating tumor growth in bone of triple-negative breast cancer. This effect was blocked by zoledronic acid (Wright et al. 2017). This paradox is not constrained to ER+ breast cancer as androgen deprivation similarly promotes prostate cancer bone metastasis in an experimental system (Schneider et al. 2005; Ottewell et al. 2014b). Few mechanistic studies have analyzed the relationship behind hormone status and bone metastasis. Thus far, these studies have only shown higher expression on RANKL on bone marrow-derived cells from menopausal women versus pre-menopausal (Eghbali-Fatourehchi et al. 2003) and that animals with experimentally-induced osteoporosis by ovariectomy or mir-34 knockout suffer from increased bone metastasis (Krzyszinski et al. 2014). Collectively, these data suggest that increased osteoclastic bone resorption by hormone deprivation therapy (both androgen and estrogen) can induce a high bone turnover state that may fuel tumor growth in bone. Thus, it is very important to monitor skeletal health and prevent bone loss when treating cancer patients with these hormonal therapies.

Numerous interactions that have been studied in models of bone metastasis have also resulted in late-stage clinical trial failures. For example, a Phase 3 trial of an Endothelin A (ET-1) antagonist in metastatic prostate cancer showed no effect on bone metastatic progression (Carducci et al. 2007), despite ET-1 broad experimental proof for the role of ET-1 in promoting both osteoblast and prostate cancer growth (Nelson et al. 1995; Yin et al. 2003). This was due to the fact that the Phase 3 trial was designed with overall survival as a primary endpoint, rather than using a bone-specific endpoint, such as bone metastases development. Animal studies clearly showed that endothelin A blockade had bone-specific effects, rather than effects on overall survival. The primary endpoints of the Phase 3 trial were not consistent with mechanisms derived from animal models and underscore the fact that clinical trial endpoints should be chosen based on mechanisms derived from preclinical studies. Interestingly, a secondary effect of therapeutic inhibitors of the Inhibitor of Apoptosis (IAP) proteins demonstrated that while these may be effective in killing tumor cells, these therapies also stimulated the NF- κ B pathway in osteoclasts, thereby promoting bone metastasis (Yang et al. 2012) and highlighting the importance of considering metastasis-related side effect of new cancer-targeting therapies.

Conclusions and future directions

Research into the molecular mechanisms of bone metastasis has significantly enhanced our understanding of the disease, both in patients and experimental models. This has refined and redirected focus from studying bone seeding to understanding that survival via dormancy and the exit from dormancy are also crucial processes to study. Unfortunately, this has also revealed a limitation in the divergence between clinical and experimental studies – current mouse models of bone metastasis have not been developed that accurately replicate the natural history of clinical progression. Due to the lack of good models of dormancy and the challenge in developing financially viable clinical trials relevant to the prevention of bone metastasis, no therapies have been developed to target dormancy *per se*. This is particularly important as targeting of dormancy might be the most optimal method to decrease the overall incidence of bone metastasis. In future efforts to develop relevant mouse models of bone metastasis seeding, dormancy and exit, we suggest that a closer relationship be made between HSC and bone metastasis research, with discoveries from one field applied to the other.

In addition to this shortcoming in the experimental research, considerable progress must also be made in clinical research. Given that breast and prostate cancer patients are the most severely affected by bone metastasis, a more thorough understanding of how estrogen and androgen interact with both bone-resident tumor cells and endogenous stromal cells during hormone therapies must be achieved. In addition, a greater emphasis should be placed on obtaining matched primary tumor and bone metastases specimens from patients to understand how these cells evolve during metastasis and treatment progression. Finally, if therapeutic discoveries from dormancy research are to be tested, a cost-efficient framework for prophylactic clinical trials including biomarkers to identify high risk patients and alternative bone metastasis-specific endpoints that faithfully predict future long-term outcomes will need to be developed and put into clinical practice.

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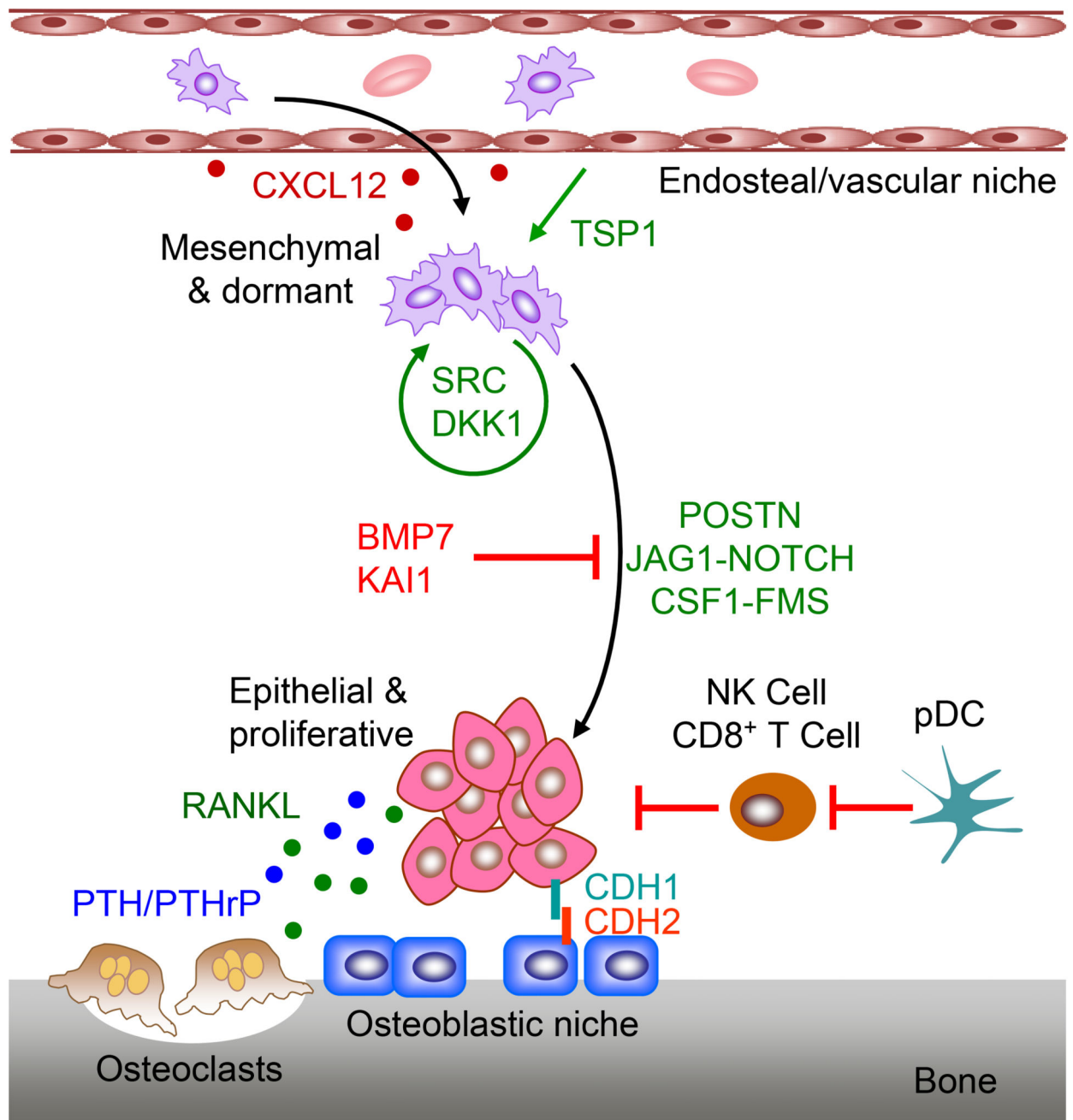


Figure 1. The balance between dormancy and outgrowth of bone-resident cancer cells

Multiple lines of research suggest that dormant cancer cells within the bone maintain a pro-survival mesenchymal state characterized by high TSP1, TGF β /BMP signaling and SRC activity as well as DKK1-suppressed Wnt activity. Upon stimulation by various stromal signaling and adhesion interactions, such as POSTN, JAG1-NOTCH, CDH1-CDH2 and CSF1-FMS, these cells enter a proliferative and epithelial state that is vulnerable to immune surveillance as well as cytotoxic agents. The processes underlying this transition remain poorly described but are thought to rely on key factors from both the endosteal niche and the osteoblastic niche. Transit and homing between these two niches appears to be regulated by

high CXCL12 levels at the endosteal site and high RANKL or PTHrP levels at the osteoblastic site.

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